UNIVERSITY OF CALIFORNIA PUBLICATIONS
IN
AGRICULTURAL SCIENCES
Vol. 2, No. 8, pp. 243-248, plate 44 September 17, 1924

MICROSPOROGENESIS OF GINKGO BILOBA L. WITH ESPECIAL REFERENCE TO THE DISTRIBUTION OF THE PLASTIDS AND TO CELL WALL FORMATION

BY
MARGARET CAMPBELL MANN
(Contribution from the Division of Genetics, University of California)

Microsporogenesis in *Ginkgo biloba* L. is especially interesting because the plastids are definitely oriented with respect to the division figures and because they are distributed so that each pollen cell receives approximately one-fourth of them. The cells are large, and both plastids and chromosomes can be observed in living cells. The 12 pairs of chromosomes are nicely separated at late prophase, and one of them is twice as large as the others. This point was previously noted by Cardiff (1906) and Ishikawa (1910).

Smears stained in aceto-carmine were used for most of this study, but smears were also fixed in Flemming's weak and chrom-acetic-urea and stained in iron-haematoxylin, Flemming's triple, and safranin and light green. The plastids are easily observed in aceto-carmine since the large starch grains resist the carmine, remaining a transparent green while the rest of the cytoplasm stains pink, and the chromosomes bright red. The starch grains stain a deep blue in iodine, but show no color in Flemming's triple or haematoxylin. With the former a layer of gentian, with the latter a layer of bluish-black cytoplasm, surrounds each starch grain. Unless one had examined the pollen mother cells before fixation, he might easily interpret the plastid-bearing area in fixed material as an unusually coarse cytoplasmic mesh. This is probably the reason why the phenomena described below have not been previously observed.

The two nuclear divisions precede cell division as in many of the higher plants. It will be seen from the account which follows that the reduction division is typical in all respects.

Before the first, or so-called heterotypic division occurs, the plastids lie between the wall and the nucleus (figs. 1 and 2). As the prophase progresses they enlarge, and some of them divide. The division is usually a simple bipartition, but some appearances suggestive of budding were seen. The cell wall is very thin at first but gradually thickens during the first division. The plastids remain between the nucleus and the wall until after the first spindle has disappeared, when they gradually move, or more probably are moved, between the two groups of late anaphase chromosomes, there forming a ring which nearly fills the space between the two nuclei (fig. 4). This position is retained until after the second division. In the late second ana- or early telophase a portion of the ring of plastids is drawn between each of the two sets of daughter nuclei (fig. 7). The ring then breaks in four places, so that one quarter of it, and consequently about a fourth of the plastids, come to surround the inner face of each nucleus (figs. 7 and 8). The outer wall now pushes inward between each of the four nuclei and finally separates the pollen cells (fig. 9). Before the inpushing of the outer wall two cell plates form at right angles to each other. The method of cell division in *Ginkgo*, then, is a combination of cell plate formation and cytokinesis. The inner portion of the wall (which lies next to the plastid-bearing area) thickens greatly while the outer wall remains unchanged. As the inner wall attains its final thickness, the plastids become generally distributed between it and the nucleus, and a new wall, staining in gentian-violet, appears about each pollen cell. The old wall stains in orange or in light green. As the pollen cells grow they burst the thin outer walls of this case, leaving an empty shell. The plastids now appear smaller, since the amount of starch in them is considerably reduced.

Juranyi (1872, pl. 31, fig. 13) figured a light band between the two daughter nuclei of the pollen mother cells of *Ceratozamia*, which is very like the position of the starch grains of *Ginkgo* at this stage. Sprecher (1907, p. 155) figured the cell plate formation of the pollen mother cells of *Ginkgo*, but neither figures nor mentions plastid or starch grain distribution. Moore (1903) figured plastids like those of *Ginkgo* in the pollen mother cells of *Pallavincinia* but does not discuss their distribution. Smith (1907) shows starch grains in the pollen

tube of *Cycas* which greatly resemble those seen in the pollen mother cells of *Ginkgo*.

The procedure described above raises a number of interesting questions. Firstly, the position of the starch grains bears a definite relation to the formation of the cell walls. They are generally distributed while the first wall is forming, and it ceases to thicken when they withdraw. They lie near the forming cell plate, and near the thick inner wall during its formation. Finally, they become generally distributed during the formation of the pollen cell wall. They are also smaller than they were during the formation of the thick inner wall of the pollen case. It seems possible that they may provide the reserve material which is utilized in wall formation.

Secondly, the method of cell wall formation differs from that common to the higher plants. This is of particular interest on account of the phylogenetic position of *Ginkgo*.

The changes of position of the starch grains are essentially the same as those noted by Terni (1914) for the chondriosomes in the spermatogenesis of *Geotriton fuscus*, and by Payne (1916) for certain scorpions. The mitochondrial mass forms the tail sheath in such spermatozoa. It would be interesting to know the fate of the plastids in spermatozoa formation of *Ginkgo*.

The similarity of behavior of the chondriosomes during cell division to that observed for the starch-filled plastids of *Ginkgo* indicates that the distributing mechanism is very similar in each case. It does not seem necessary to postulate a separate mechanism for this purpose, the forces already in action being of a type which would, it seems to me, bring about essentially the observed distribution. Whatever the force or forces may be by which the chromosomes are distributed during reduction, the direction of movement of the chromosomes shows the direction in which these forces act. One would expect that a large number of movable cytoplasmic structures or inclusions would be equally distributed between the cell wall and the nucleus, if in- and out-going currents maintained an equilibrium during the early prophase. After the spindle has formed, and the cell is a bipolar structure, the forces (which we may think of as currents) move toward the poles, and presumably back toward the equator. The changes of position of the plastids at this stage indicate such lines of force. As the nuclei grow, apparently by taking in fluid, their increase in size would also tend to force the plastids out of the polar and into the equatorial

regions. The barrel-shaped spindle would hold them in ring formation. When the nuclei have attained their final size, the plastids, now filled with starch, form two rows with the cell plate between them. With the formation of the second spindles this line of division is obliterated. It is possible either that the processes involved in cell plate formation produce the line of division, or that it results from the action of the opposing forces concerned in chromosome division. In any case, the distortion of the ring at late second ana- and early telophase might result from the pull of the same forces which separated the chromosomes. The final division of the plastids into four groups may depend somewhat upon the invaginations which give rise to the lateral walls of the pollen case. In the pollen cell the plastids again revert to the position which they had at early prophase of the pollen mother cell.

LITERATURE CITED

CARDIFF, I. D.

1906. A study of synapsis and reduction. Bull. Torr. Bot. Club, vol. 33, pp. 271–303.

ISHIKAWA, M.

1910. Uber die Zahl der Chromosomen von *Ginkgo biloba* L. The Bot. Mag., Tokyo, vol. 24, pp. 225–226.

JURANYI, L.

1872. Uber den Bau und die Entwickelung des Pollens bei *Ceratozamia longifolia* Miq. Jahrb. f. Wiss. Bot., vol. 8, pp. 382–400, pls. 31–34.

MOORE, A. C.

1903. The mitosis in the spore mother cell of Pallavicinia. Bot. Gaz., vol. 36, pp. 384–388.

PAYNE, F.

1916. Germ cells of Gryllotalpa. Jour. Morph., vol. 28, pp. 287–327.

SMITH, F. G.

1907. Morphology of the trunk and development of microsporangium of Cycas. Bot. Gaz., vol. 43, pp. 187–204, pl. 10.

SPRECHER, A.

1907. Le *Ginkgo biloba* L. (Geneva) 207 pp.

TERNI, T.

1914. Condriosomi, idiozoma e formazioni periidiozomiche nella spermatogenesi degli Anfibrii. (Ricerche sul *Geotriton fuscus.*) Arch. f. Zellf., vol. 12, pp. 1–96, pls. 1–7.

WILSON, E. B.

1916. The distribution of the chondriosomes to the spermatozoa of scorpions. Science, vol. 43, p. 539.

PLATE 44

The drawings are semi-diagrammatic. They were made with a camera lucida, using a 4 mm. dry objective and a number 18 Zeiss compensating ocular. The chromosomes and nuclei are simply outlined, the plastids at the upper focus are in gray wash with a stippled margin, while the lower ones are left white. The drawings show the position of the plastids at successive stages in microsporogenesis.

1. Polar view of late first prophase showing 12 pairs of chromosomes, one of which is about twice the size of the others.

2. Lateral view of same stage showing that the spindle area is largely free of plastids. Most of the plastids are near the poles.

3. Lateral view of late anaphase showing movement of plastids from poles toward equator of cell.

4. Late telophase. The plastids are now arranged in a double ring at the equator.

5. Late second prophase. The plastids are so closely massed that ring formation cannot be distinguished.

6. Late second anaphase. The ring of plastids is being distorted.

7. Second telophase. The ring is now breaking into four segments, one group lying about the inner face of each of the four nuclei.

8. Early cell division. The dotted lines show cell plate. The outer wall is pushing inward between each of the four nuclei.

9. Cell division completed.

1
2
3
4
5
6
7
8
9